

**CDPK5 AND MSP1 GENE MODULATION AND *IN VITRO* PARASITE
INHIBITION BY MCL OR ITS COMBINATION WITH ARTEMISININ**

**OKAFOR, ESTHER OGECHI
(21PCP02254)**

B.Sc Biochemistry, Federal University Otuoke, Bayelsa State, Nigeria

AUGUST 2023

**CDPK5 AND MSP1 GENE MODULATION AND *IN VITRO* PARASITE
INHIBITION BY MCL OR ITS COMBINATION WITH ARTEMISININ**

BY

**OKAFOR, ESTHER OGECHI
(21PCP02254)**

B.Sc Biochemistry, Federal University Otuoke, Bayelsa State, Nigeria

**A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE
STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR
THE AWARD OF MASTER OF SCIENCE, (M.Sc) IN BIOCHEMISTRY IN
THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF SCIENCE AND
TECHNOLOGY, COVENANT UNIVERSITY, OTA, OGUN STATE,
NIGERIA**

AUGUST 2023

ACCEPTANCE

This is to attest that this dissertation is accepted in partial fulfilment of the requirements for the award of Master of Science (M.Sc.) degree in Biochemistry in the Department of Biological Sciences, College of Science and Technology, Covenant University Ota, Ogun State, Nigeria.

Ms. Adefunke F. Oyinloye

(Secretary, School of Postgraduate Studies)

Signature and Date

Prof. Akan B. Williams

(Dean, School of Postgraduate Studies)

Signature and Date

DECLARATION

I, OKAFOR, ESTHER OGECHI (21PCP02254) declare that I carried out this research under the supervision of Dr. Titilope M. Dokunmu of the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Nigeria. I attest that the dissertation has not been presented wholly or partially for the award of any degree elsewhere. All the sources of materials and scholarly publications used in the dissertation have been duly acknowledged.

OKAFOR, ESTHER OGECHI

Signature and Date

CERTIFICATION

We certify that this dissertation titled “**CDPK5 AND MSP1 GENE MODULATION AND *In vitro* PARASITE INHIBITION BY MCL OR ITS COMBINATION WITH ARTEMISININ**” is an original work carried out by **OKAFOR, ESTHER OGECHI (21PCP02254)** in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria, under the supervision of Dr. Titilope M. Dokunmu. We have examined and found the work acceptable as part of the requirements for the award of a degree of Master of Science (M.Sc.) in Biochemistry.

Dr Titilope M. Dokunmu
(Supervisor)

Signature and Date

Prof. Israel S. Afolabi
(Head of Department)

Signature and Date

Prof. Adedoyin Igunnu
(External Examiner)

Signature and Date

Prof. Akan B. Williams
(Dean, School of Postgraduate Studies)

Signature and Date

DEDICATION

This report is dedicated to the almighty God, the giver, and sustainer of life, for His unconditional love and mercy granted to me through this project. And to my parents Mr. and Mrs. Okafor and siblings for their constant love and support.

ACKNOWLEDGEMENTS

I want to begin by thanking the Lord God Almighty for His mercies, grace, and strength throughout this work.

I would like to thank the Chancellor of Covenant University, Dr. David Oyedepo for his visionary and exemplary leadership that established this great citadel of learning and has continued to inspire me to work towards achieving my set goals. I also thank you sir for your continued prayers and prophetic declarations over Covenant University students as a whole.

I would like to acknowledge the faculty and staff of the Biochemistry department most especially the head of the department Prof. Israel S. Afolabi, and the Biochemistry Post graduate coordinator Dr. Titilope M. Dokunmu for their leadership roles. I would like to thank my supervisor, Dr. T.M. Dokunmu, for guiding me through this work. May God almighty richly bless you and your families.

I am highly indebted to all the members of Covenant University Bioinformatics Research Cluster (CUBRe); especially Prof. Ezekiel Adebisi, for funding my M.Sc. My sincere appreciation goes to the World Bank-Funded Covenant Applied Informatics and Communications Africa Center of Excellence (CApIC-ACE) for funding my research project and the management, faculty and students at CApIC-ACE.

I also appreciate all the Faculty members of the Biochemistry unit for their comments, remarks, corrections and helpful tips during the presentation of this work. Also, to the Management and Staff of the Department of Biochemistry, Covenant University for providing an enabling environment needed to carry out this research. I also say a big thank you to Prof. O.O. Ajani in Chemistry Department, Covenant University for all the support and assistance.

I definitely would not forget to express my deepest appreciation to my parents, Mr. and Mrs. Okafor for their support and encouragement financially, morally and spiritually. I pray that God Almighty continues to protect you and bless the works of your hands. I would also say a big thank you to my siblings, best friend (Mercy) and boyfriend (Oluwatimileyin) for their love and support. May God Almighty continue to protect you and I wish you success in your endeavors.

TABLE OF CONTENTS

CONTENTS	PAGES
COVER PAGE	ii
ACCEPTANCE	iii
DECLARATION	iv
CERTIFICATION	v
DEDICATION	vi
ACKNOWLEDGEMENTS	vii
LIST OF TABLES	xi
LIST OF FIGURES	xii
ABBREVIATIONS	xiii
ABSTRACT	xiv
CHAPTER ONE	1
INTRODUCTION	1
1.1 Background to the study	1
1.2 Statement of Problem	4
1.3 Research Questions	4
1.4 Aim and Objectives of the Study	4
1.5 Justification for the Study	5
1.6 Scope of the Study	5
CHAPTER TWO	6
LITERATURE REVIEW	6
2.1 <i>Plasmodium falciparum</i> Lifecycle and Development	6
2.2 Drug Targets Under Study	10
2.2.1 <i>Plasmodium falciparum</i> Calcium-dependent Protein Kinases (<i>PfCDPK5</i>)	10
2.2.2 Merozoite Surface Protein (MSP)	11
2.3 Classes of Antimalarials	14
2.3.1 Quinolones	16
2.3.2 Artemisinin	18
2.4 Antimalarial Drug Resistance	20
2.5 Antimalarial Drug Development	24
CHAPTER THREE	26
MATERIALS AND METHODS	26

3.1 Materials	26
3.1.1 Equipment	26
3.1.2 Reagents	26
3.2 Methodology	27
3.2.1 Study Design	27
3.2.2 Inclusion/Exclusion criteria	27
3.2.3 Study population	27
3.2.4 Study subject	28
3.2.5 Sample Collection and Preparation	28
3.2.6 Maintaining culture media and preparing the blood smear	31
3.2.6.3 Cryopreservation	31
3.2.7 Drug sensitivity testing	32
3.2.8 Relative Gene Expression Study	33
3.3 Methods of statistical analysis	40
CHAPTER FOUR	63
RESULTS	63
4.1 <i>In vitro</i> Study	63
4.1.1 Parasite Clearance	63
4.1.2 <i>In vitro</i> antiplasmodial activity of MCL and combination on wild strain <i>Plasmodium falciparum</i> (3D7)	45
4.2 Relative gene expression study	48
4.2.1 Relative gene expression (Δ Ct) of <i>PfMSP1</i> in ART, MCL and ART+MCL per day	48
4.2.2 Relative gene expression (Δ Ct) of <i>PfCDPK</i> in ART, MCL and combination per day	48
CHAPTER FIVE	51
DISCUSSION	51
5.1 <i>In vitro</i> Study	51

5.2 Relative Gene Expression Study	52
CHAPTER SIX	54
CONCLUSION AND RECOMMENDATION	54
6.1 Summary	54
6.2 Conclusion	54
6.3 Contributions to Knowledge	55
6.4 Recommendations	55
REFERENCES	56
APPENDICES	67

LIST OF FIGURES

FIGURES	TITLE OF FIGURES	PAGES
2.1	The lifecycle of the <i>Plasmodium</i> parasite in mosquitoes and the human host	8
2.2	Stages of <i>P. falciparum</i>	8
2.3	Core Secretory Organelles of the Merozoite	9
2.4	Development of <i>Plasmodium falciparum</i> gamatocytes	9
2.5	Phases of Erythrocytic Invasion	13
2.6	Structure-Activity Relationships of the Quinolone core	17
2.7	Quinoline and quinolone antibiotics	17
2.8	Structures of artemisinin and several representative derivatives	19
2.9	Chemical structure of MCULE-7146940834	25
4.1	Dose-Response Curve for Artemisinin	46
4.2	Dose Response Curve for MCL + ART.	46
4.3	Dose-Response Curve for MCL.	47
4.4	Relative gene expression (Δ Ct) of <i>PfMSP1</i> in ART, MCL and combination per day	49
4.5	Relative gene expression (Δ Ct) of CDPK 1 in ART, MCL and combination per day	49

LIST OF TABLES

TABLES	TITLE OF TABLES	PAGES
2.1	Classifying Antimalarials by the Life Cycle Stage	15
2.2	Drug-Resistant Antimalarials	22
2.3	Drugs and Resistant Genes	23
3.1	Reagents Required for the Preparation of Culture Medium	30
3.2	Drug Concentrations for Sensitivity Assay	30
3.3	Grouping of the cultured plate for dosing	36
3.4	One-step reverse transcription PCR (RT-PCR) reaction mix for <i>PfMSP1</i> and <i>PfCDPK5</i>	37
3.5	One-step reverse transcription PCR (RT-PCR) reaction condition for <i>PfMSP1</i> and <i>PfCDPK5</i> genes	38
3.6	Primer sequence of target genes	39
4.1	The parasite clearance rate of MCL + ART at different concentrations	64
4.2	The parasite clearance rate for Artemisinin at different concentrations	44
4.3	The parasite clearance rate of MCL at different concentrations	44
4.4	ANOVA table of the effect of the type of drug and days of administration on the expression of <i>PfMSP1</i>	50
4.5	ANOVA table of the effect of the type of drug and days of administration on the expression of <i>PfCDPK5</i>	50

ABBREVIATIONS

ART	artemisinin
BSA	Bovine Serum Albumin
CQ	chloroquine
DMSO	dimethyl sulfoxide
DNA	Deoxyribonucleic acid
HEPES	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid
IC ₅₀	50% inhibitory concentration
MSP2	Merozoite Surface Protein 2
MW	molecular weight
PCR	Polymerase chain reaction
<i>PfCRT</i>	<i>P. falciparum</i> Chloroquine esistance transporter gene
<i>PfMDR1</i>	<i>P. falciparum</i> multidrug resistance gene 1
<i>PfMRP</i>	<i>P. falciparum</i> multidrug resistance-associated protein
<i>PfMSP1</i>	Merozoite Surface Protein 1
<i>PfNHEL</i>	<i>Plasmodium falciparum</i> sodium hydrogen exchanger
RBC	red blood cell
RPMI	Roswell Park Memorial Institute
RSA	ring-stage survival assay
RT	room temperature
WBC	white blood cell
WHO	World Health Organization

ABSTRACT

Recent treatment failures in artemisinin-based combination therapy (ACT) have raised concerns about its efficacy against malaria and emphasize the need to discover new treatment targets and resistance-free drugs. A small molecule (MCULE-7146940834 - MCL) showed inhibitory potential against *Plasmodium falciparum*, targeting gene families crucial for red blood cell invasion *in silico* but has not been validated *in vitro*. This study assesses MCULE-7146940834 *in vitro*, evaluating its inhibitory concentration (IC₅₀) and gene modulation effects on Merozoite Surface Protein 1 (*PfMSP1*) and Calcium-dependent Protein Kinase (*PfCDPK5*), both independently and in combination with Artemisinin (ART). *PfMSP1* facilitates the attachment and binding of the merozoite to the host RBC, while *PfCDPK5*, facilitates the secretion of invasion-related proteins and motor function to drive penetration making them promising antimalarial drug targets. *Plasmodium falciparum* derived from field isolates and the 3D7 strain were cultured within O+ human red blood cells. This cultivation occurred in RPMI 1640 medium supplemented with 10% heat-inactivated human serum, 25 mM HEPES buffer, and 50 µg/ml penicillin-streptomycin. The entire process was carried out under controlled conditions of 5% CO₂ at 37°C. Serially diluted drugs of ART, ART+MCL, and MCL were administered to 96-well microtitre plates, over 72 hours, with doses incrementing by a factor of 10 from 0 to 100µM. This procedure adhered to the WHO micro-test protocol and involved incubating the substances with parasite culture medium samples at a parasitemia level of 0.2% and a haematocrit of 4% for the same duration. The evaluation of parasitemia was performed microscopically using Giemsa-stained smears. RNA from cultured samples, pre- and post-treatment, was extracted, quantified, and analysed by real-time polymerase chain reaction using primers specific for the *PfMSP1* and *PfCDPK5* genes and *PfGAPDH* as an internal reference gene. All assays were carried out in duplicates and analyzed using graph pad prism software at $p < 0.05$. The outcomes from the analysis of half maximal inhibitory concentrations (IC₅₀) using linear regression demonstrated an *in vitro* IC₅₀ value of 24.68 µM for MCL, and a value of 5.006 µM for the combination of MCL with artemisinin. Relative gene expression (ΔC_t) shows increased expression of *PfMSP1* and *PfCDPK5* relative to *PfGAPDH*. These results demonstrated that the MCULE-7146940834 holds promise as a potential candidate for antimalarial drug development, making it a valuable hit compound for subsequent optimization.